## WHAT IS CLAIMED IS:

- 1. A single-layer protein system comprising:
- (i) a protein;
- (ii) two ligands I and II which bind with different affinities at the same binding site of said protein, said ligand I being the low affinity ligand and said ligand II being the high affinity ligand; and
- (iii) a molecule that recognizes the low affinity
  ligand I,

wherein in said single-layer protein system the high affinity ligand II is buried within the binding site of the protein (i) and the low affinity ligand I is covalently bound to the protein and associated with the molecule (iii) that recognizes it.

- A single-layer protein system according to claim
   wherein the molecule (iii) is labeled with high affinity
   ligand II.
- 3. A multilayer protein system comprising two or more layers according to claim 2.
- 4. A multilayer protein system according to claim 3 wherein in the last layer molecule (iii) is not labeled with high affinity ligand II.
- 5. A single-layer or multilayer protein system according to claim 3, wherein the protein (i) is an avidin-

type molecule selected from the group comprising native eggwhite avidin, recombinant avidin, deglycosylated avidins,
bacterial streptavidin, recombinant streptavidin, truncated
streptavidin and other derivatives of said avidin-type
molecules; the low affinity ligand I is HABA or a HABA
derivative, the high affinity ligand II is biotin, and the
molecule (iii) that recognizes ligand I is an anti-HABA
antibody or a biotinylated anti-HABA antibody.

- 6. A single-layer or multilayer protein system according to claim 3, wherein the protein (i) is antidinitrophenyl (DNP)-antibody; the low affinity ligand I is trinitrobenzene (TNP) or mononitrobenzene (MNP), the high affinity ligand II is DNP and the molecule (iii) that recognizes ligand I is optionally DNP-tagged anti-MNP or anti-TNP antibodies.
- 7. A single-layer or multilayer protein system according to claim 3, formed on a substrate selected from the group consisting of gold, silicium, polystyrene.
- 8. A multilayer protein system according to claim 3 comprising 5-6 layers.
- 9. A method for assembling a multilayer protein system according to claim 3, which comprises the steps of:
- (a) covalently binding said low affinity ligand I to said protein (i), thus obtaining a low affinity ligand I-

- protein (i) complexin which said ligand I is buried within the binding site of said protein (i) and is thus not available for interaction with other molecules that recognize it;
- (b) reacting the high affinity ligand II or a compound containing said high affinity ligand II with the low affinity ligand I-protein (i) complex of step (a) above, whereby low affinity ligand I is expelled from within the binding site to the periphery but remains covalently bound to protein (i) and high affinity ligand II is associated to, and buried within, the binding site of protein (i);
- (c) reacting the low affinity ligand I-protein(i)-high affinity ligand II complex of step (b) with a molecule (iii) that recognizes and binds to low affinity ligand I and is labeled with high affinity ligand II; and
- (d) reacting the protein system of step (c) with low affinity ligand I- protein (i) complex as in step (b) above, and repeating steps (c) and (d) as desired.
- according to claim 1, wherein the protein (i) is an avidintype molecule selected from the group comprising native eggwhite avidin, recombinant avidin, deglycosylated avidins, bacterial streptavidin, recombinant streptavidin, truncated streptavidin and other derivatives of said avidintype molecules; the low affinity ligand I is HABA or a HABA

derivative, the high affinity ligand II is biotin, and the molecule (iii) that recognizes ligand I is an anti-HABA antibody or a biotinylated anti-HABA antibody.

- according to claim 1, wherein the protein (i) is antidinitrophenyl (DNP)-antibody; the low affinity ligand I is trinitrobenzene (TNP) or mononitrobenzene (MNP), the high affinity ligand II is DNP and the molecule (iii) that recognizes ligand I is optionally DNP-tagged anti-MNP or anti-TNP antibodies.
- 12. A single-layer or multilayer protein system according to claim 1, formed on a substrate selected from the group consisting of gold, silicium, polystyrene.
- 13. A method for assembling a single-layer protein system according to claim 1, which comprises the steps of:
- (a) covalently binding said low affinity ligand I to said protein (i), thus obtaining a low affinity ligand I-protein (i) complex in which said ligand I is buried within the binding site of said protein (i) and is thus not available for interaction with other molecules that recognize it;
- (b) reacting the high affinity ligand II or a compound containing said high affinity ligand II with the low affinity ligand I-protein (i) complex of step (a) above, whereby low affinity ligand I is expelled from within the

binding site to the periphery but remains covalently bound to protein (i) and high affinity ligand II is associated to, and buried within, the binding site of protein (i);

- (c) reacting the low affinity ligand I-protein(i)-high affinity ligand II complex of step (b) with a molecule (iii) that recognizes and binds to low affinity ligand I and is labeled with high affinity ligand II; and
- (d) reacting the protein system of step (c) with low affinity ligand I- protein (i) complex as in step (b) above, and repeating steps (c) and (d) as desired.